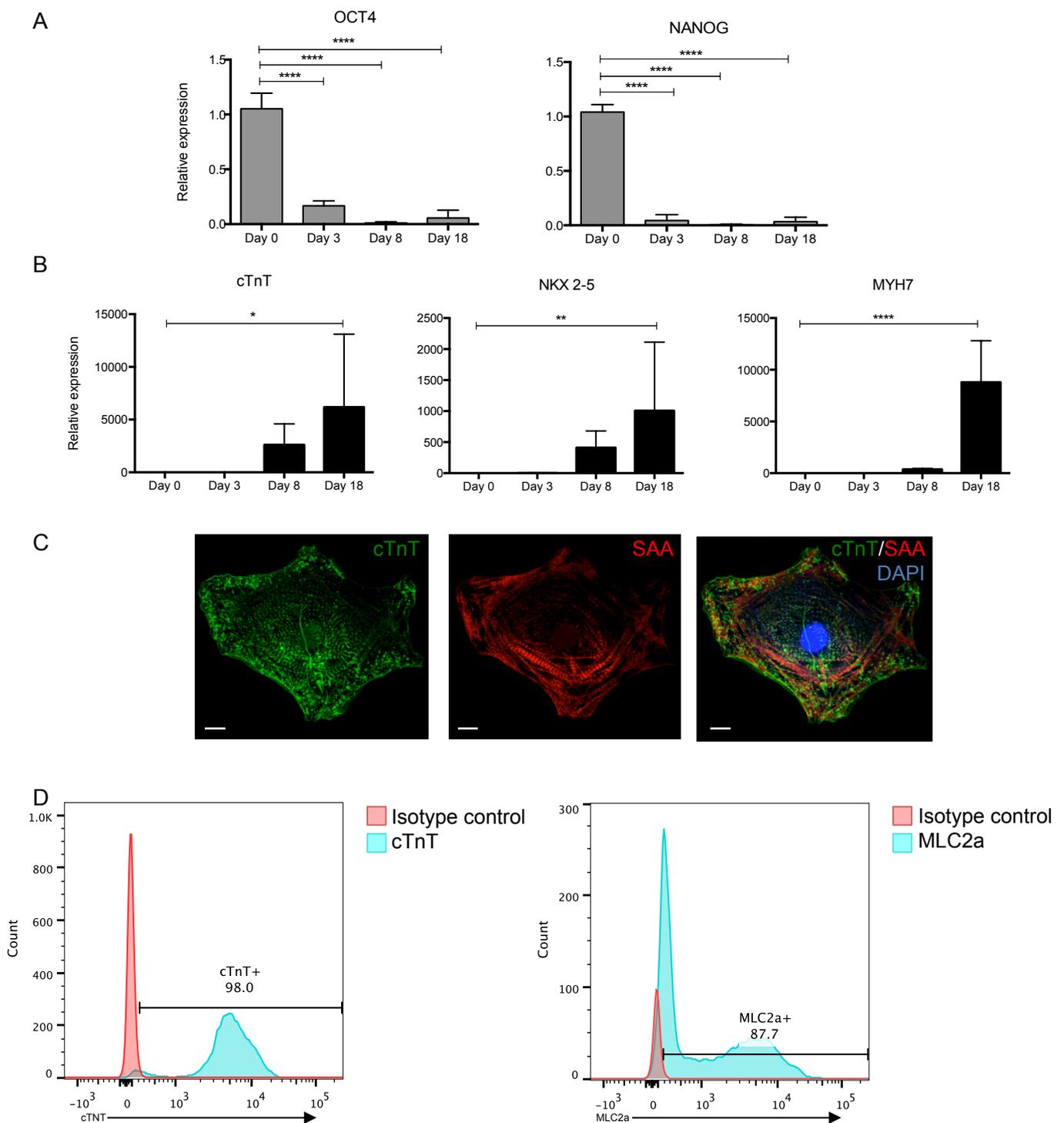


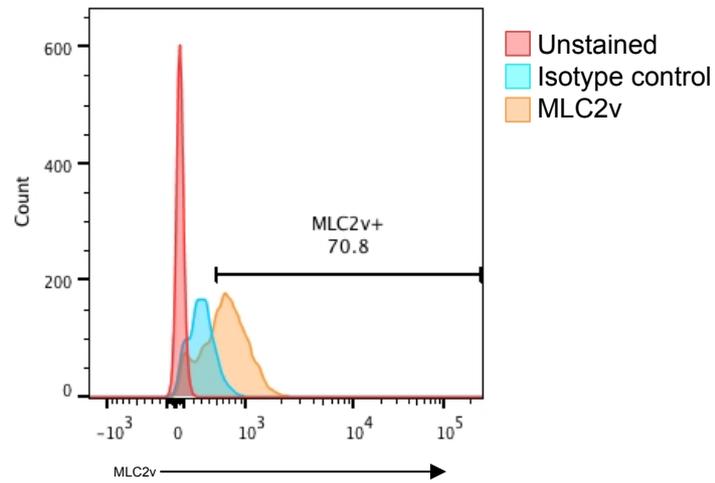
**MODELING DOXORUBICIN-INDUCED CARDIOTOXICITY IN  
HUMAN PLURIPOTENT STEM CELL DERIVED-  
CARDIOMYOCYTES**

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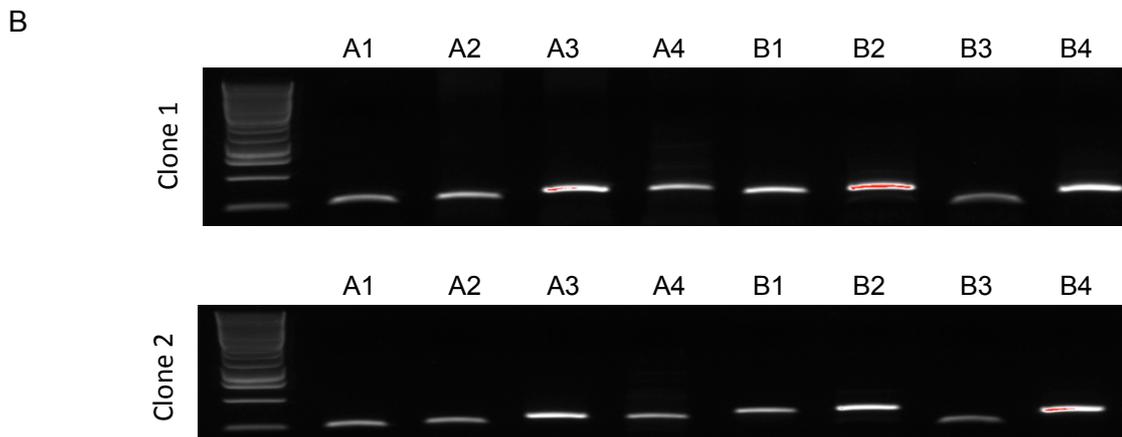


**Supplementary figure 1: Characterization of hPSC-derived cardiomyocytes (NKX2-5<sup>eGFP/w</sup> cell line).** A) Relative gene expression levels of pluripotency markers (A) and cardiac markers (B). The mean Ct values of duplicate measurements were normalized against the values obtained for  $\beta$  actin for the same sample. After normalization, the means of three independent experiments were plotted. Data represent the mean  $\pm$  SD. C) Immunostaining of hPSC-derived CMs with cTnT and SAA antibodies followed by counterstaining with DAPI. D) Flow cytometry analysis of cTnT and MLC2a expression. cTnT: cardiac Troponin T, MYH7: myosin heavy chain beta; SAA: sarcomeric actinin alpha, MLC2a: myosin light chain 2a. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\*\* $p < 0.0001$ . Scale bar: 10  $\mu$ m



**Supplementary figure 2: MLC2v expression in hPSC-derived cardiomyocytes (hES3 cell line).** Flow cytometry analysis MLC2v expression revealed that a majority of the cardiomyocytes expressed the ventricular marker MLC2v, indicating that these cell are predominantly ventricular-like.

A		
A1	TTCAGAGTTGAGGTTACCAGTCCATTTAAGGTAACATGGTATAGGTTGGGGGAGTACTTGCTTTAGGATA GTTGTGATGCTAAGTGTGTTAATGTCTATAGAATA	Chr 7 Position 103002807 Score 2.603
A2	TCCCAGTTGCCAAGGAAGGCCTGGGTCTTCTTAATTTGTTTTAGTCCCAAAGCAAGTACTTACCTAAAC TGAATAGAGGCAGGAACCTATCCAGTCTAAGCTCTC	Chr 12 Position 56373518 Score 1.632
A3	AATGGCTTTTCTAAAATCCATTCATCAACCAGCAGGAAAAGACTAAAGCAAGTTCTCACCCGAGATTGG GAAGCAGAAAAGTTAATGAGCTACCTGACATTCTCTCT	Chr 2 Position 238524473 Score 1.384
A4	TCCAATCCAAACACCTCATCTCTGAATTTTACAGCCTTTTCTGGGGGAGCACTTGCTTAGGAAAGAAAC ATTCAACCTTAAACCACCAGGGCAACGGGGACAAAGCT	Chr 14 Position 77472439 Score 1.330
B1	CAGTTAGACTATAGCCCTTGTGTATTTATAGTTTCCATGAATGTGTACTAAATGATTTTGATAAACCAAGT ATTTGCATGTGAAACTTTTTCTGTCTCTAGTATCAT	Chr 3 Position 197801471 Score 1.592
B2	GAAGCACAAAATTCAGTAAGTCAAAGCAAAGATTTTCATCCCATGAGTCCAGGTTTATACATTTTCTTTGA CATGCAATGATTTTTAAGTAGTTTTATTCTTCATGTG	Chr 20 Position 53396700 Score 1.305
B3	TTTTGGGACTGGAAAATACAGAAGACTCCTTGGTTTATACATGGTCTTGTAAGAGAATCAGTACTTACAG CACTATTGCAATTCCAAGTATTGAAGCAATCGCAGGT	Chr 6 Position 116560209 Score 1.278
B4	GATCTTTATATTAAGGATTTACTCACCTAGAATCTCATCAAAGATTTGTACAAACCAGGAACAAAAGTG ACTTCCCTATAGTTAATGCCAACATCTTCATCGTAA	Chr 17 Position 38572684 Score 1.095



**Supplementary figure 3: Analysis of the main potential off-target sites of Top2b CRISPR-Cas9.** A) Potential off-target sites of Top2b-CRISPR gRNA1 (A1 to A4) and gRNA2 (B1 to B4). B) PCR amplification with primers specific to sequences A1 to B4. PCR products were purified and subjected to Sanger sequencing.

## Supplementary Table 1: Pathways up-regulated by doxorubicin treatment

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Pathway
KEGG_Ribosome
KEGG_ECM Receptor Interaction
KEGG_Lysosome
KEGG_Oxidative Phosphorylation
KEGG_Cell Adhesion Molecules CAM
KEGG_Alzheimers Disease
KEGG_Glutathione Metabolism
KEGG_Other Glycan Degradation
KEGG_Parkinsons Disease
KEGG_Cardiac Muscle Contraction
KEGG_Huntingtons Disease
KEGG_Complement And Coagulation Cascades
KEGG_Dilated Cardiomyopathy
KEGG_Valine Leucine And Isoleucine Degradation
KEGG_Focal Adhesion
KEGG_Arginine And Proline Metabolism
KEGG_Hypertrophic Cardiomyopathy
KEGG_Systemic Lupus Erythematosus
KEGG_Glycosaminoglycan Degradation
KEGG_Arrhythmogenic Right Ventricular Cardiomyopathy
KEGG_Vibrio Cholerae Infection
KEGG_Hematopoietic Cell Lineage
KEGG_Biosynthesis Of Unsaturated Fatty Acids
KEGG_Glycolysis Gluconeogenesis
KEGG_Citrate Cycle TCA Cycle
KEGG_Galactose Metabolism
KEGG_Antigen Processing And Presentation
KEGG_Glyoxylate And Dicarboxylate Metabolism
KEGG_Metabolism Of Xenobiotics By Cytochrome P450
KEGG_Viral Myocarditis
KEGG_Drug Metabolism Cytochrome P450
KEGG_Fatty Acid Metabolism
KEGG_Glycosylphosphatidylinositol GPI Anchor Biosynthesis
KEGG_Protein Export
KEGG_Propanoate Metabolism
KEGG_Beta Alanine Metabolism
KEGG_Leukocyte Transendothelial Migration
KEGG_Butanoate Metabolism
KEGG_Pyruvate Metabolism
KEGG_Pentose Phosphate Pathway
KEGG_Arachidonic Acid Metabolism

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## Supplementary Table 2: Pathways down-regulated by doxorubicin treatment

Pathway
KEGG_Ubiquitin Mediated Proteolysis
KEGG_RNA degradation
KEGG_Spliceosome
KEGG_Wnt Signaling Pathway
KEGG_Prostate Cancer
KEGG_MAPK Signaling Pathway

**Supplementary Table 3: List of Realtime PCR Primers Used**

<b>Gene name</b>	<b>Direction</b>	<b>Primer sequence</b>
<i>MYH7</i>	Forward	CTCGCCAGAATGGAGTACAAA
<i>MYH7</i>	Reverse	CTTCATCCAGGGCCAATTCT
<i>NKX2.5</i>	Forward	CTACGGTTATAACGCCTACCC
<i>NKX2.5</i>	Reverse	CGAAGTTCACGAAGTTGTTGTT
<i>cTnT</i>	Forward	CAAAGGAGGCTGAAGATGGC
<i>cTnT</i>	Reverse	CAAAGTGAGCCTCGATCAGC
<i>β actin</i>	Forward	GGCATGGGTCAGAAGGATTC
<i>β actin</i>	Reverse	CACACGCAGCTCATTGTAGAAG
<i>hOCT4</i>	Forward	TCTTTCCACCAGGCCCCCGGCTC
<i>hOCT4</i>	Reverse	TGCGGGCGGACATGGGGAGATCC
<i>Nanog</i>	Forward	CATGAGTGTGGATCCAGCTTG
<i>Nanog</i>	Reverse	CCTGAATAAGCAGATCCATGG
<i>hTERT</i>	Forward	TGCGGCCGATTGTGAAC
<i>hTERT</i>	Reverse	CCTCTTTTCTCTGCGGAACGT